Basic Mechanisms of Asthma

Homer A. Boushey and John V. Fahy

Cardiovascular Research Institute, University of California San Francisco, San Francisco, California

Results of studies of the epidemiology, physiology, histopathology, and cell biology of asthma have revised our conception of the disease. Epidemiologic studies have shown asthma to be an important cause of death, suffering, and economic hardship. Physiologic studies have shown that asthma is a chronic illness characterized by persistent bronchial hyperreactivity. Histopathologic studies have shown characteristic changes: epithelial damage, deposition of collagen beneath the basement membrane, eosinophilic and lymphocytic infiltration, and hypertrophy and hyperplasia of goblet cells, submucosal glands, and airway smooth muscle. Studies of the functions of cells in the airway mucosa suggest that asthma may be fundamentally mediated by a difference in the type of lymphocyte predominating in the airway mucosa but may also involve complex interactions among resident and migratory cells. Asthma may thus result from sensitization of a subpopulation of CD4+ lymphocytes, the Th2 subtype, in the airways. These lymphocytes produce a family of cytokines that favor IgE production and the growth and activation of mast cells and eosinophils, arming the airways with the mechanisms of response to subsequent reexposure to the allergen. This conceptual model has stimulated research along lines that will almost certainly lead to powerful new treatments, and it has already put current therapies in a new light, clarifying the role of antiinflammatory agents, especially of inhaled corticosteroids. This conceptual model has some limitations: it ignores new evidence on the role of the mast cell in producing cytokines and depends on results of studies of the effects of inhalation of allergen, although most asthma exacerbations are provoked by viral respiratory infection. Preliminary studies suggest that viral infection and allergen inhalation may involve the activation of different pathways, with viral infection activating production of cytokines by airway epithelial cells. Similar study of the mechanisms activated by inhalation of air toxics may provide important clues as to how they might induce or exacerbate asthma. — Environ Health Perspect 103(Suppl 6):229–233 (1995)

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Introduction

The conception of the pathogenesis of disease drives far more than the thoughts on what treatments might be most effective. It affects also the resources allocated to research and care, the direction of the research undertaken, and even the sympathy extended and the public policies adopted to protect those afflicted. For asthma, the past 10 or 15 years have brought about a dramatic change in the conception of pathogenesis that has in turn led to the proposal of previously unconsidered mechanisms by which other inhaled materials might worsen the condition.

Medicine's conception of the pathogenesis of asthma has evolved over two millennia, for the disease was known to Hippocrates. The word *asthma* is derived from the ancient Greek for panting, and even the ancient world recognized that asthma could cause death by suffocation (*1*). In the 11th century, Moses Maimonides recorded that death from asthma could occur "should the rules of management go unheeded and one's desires and habits be followed indiscriminately." In the 18th century, Thomas Watson described to the Royal Society his postmortem findings of an asthma victim, commenting on the overinflation of the lungs even after their removal from the thorax (*1*).

Despite this early and consistent recognition of asthma as a serious, potentially lethal disorder, the conception of asthma underwent a change in the 19th century, perhaps because physicians were so regularly confronted with patients dying from inexorably progressive tuberculosis. Laennec, inventor of the stethoscope, stated that asthma confers a prospect for long life and freedom from other diseases. Oliver Wendell Holmes described asthma as "a slight ailment that prolongs longevity." Even William Osler said, "The asthmatic pants into old age" (*1*).

The general view held through the middle of this century was that asthma is a mild disease, possibly of psychosomatic origin, requiring occasional or regular bronchodilator therapy, with corticosteroids being reserved for severe acute attacks or for the rare patient with chronic severe asthma.

The observations responsible for changing this concept of asthma, and ultimately for the emergence of our current concept of asthma's pathogenesis, derived from studies of the epidemiology, physiology, and pathology of the condition.

Increasing Morbidity and Mortality of Asthma

That asthma is an important cause of mortality was dramatically reestablished by sharp increases in asthma deaths, especially in boys and young men, in the United Kingdom in the mid 1960s and in New Zealand in the late 1970s (*2,3*). These epidemics of asthma deaths caused other countries to analyze their health statistics; by the late 1980s, all developed western countries with reliable statistics reported an increase in asthma deaths (*4,5*). Recent reports show these trends to have continued. The Centers for Disease Control reported that asthma deaths increased 46% from 1980 to 1989 (*6*). This increase was shared by all races and age groups, but was disproportionately greater in blacks and in children. Geographic analysis showed disproportionate increases in asthma deaths in children in Chicago and New York City, emphasizing the greater risk of death from asthma for the urban poor (*7*).

The increase in mortality may reflect a true increase in the prevalence of asthma. A careful review of the diagnoses made at all physician contacts in Olmsted County in Minnesota, a county whose demographics resemble those of the United States, showed a 55% increase in the incidence of asthma from 1964 to 1983 (*8*).

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Address correspondence to Dr. Homer A. Boushey, Cardiovascular Research Institute, Box 0130, University of California San Francisco, 505 Parnassus Street, San Francisco, CA 94143-0130. Telephone (415) 476-8019. Fax (415) 476-9531.

The general—but not unanimous—view among epidemiologists is that the increase in deaths, hospitalizations, and physician visits for asthma reflect a true increase in the prevalence of the disease. The reasons for this are unclear, but most speculation centers on the changes in building insulation driven by the rising cost of energy, a reduction in the turnover of indoor air, and increased exposure to indoor pollutants, especially environmental cigarette smoke and the potent allergens of cat dander, cockroaches, and house dust mites. Well-designed epidemiologic studies in England and Sweden show that the risk of developing asthma is related to the level of house dust mites in household air (*9,10*).

As if the cost in human suffering was not enough, the economic costs of asthma are great. Using data from the National Center for Health Statistics and conservative estimates of cost, an economic evaluation of asthma placed the total cost in 1985 as $4.5 billion: $2.4 billion in direct costs for inpatient, emergency, and outpatient care, physicians' services and medications; and $2.1 billion in indirect costs for loss of work and premature death (*11*). Adjusted for inflation, this is $6.2 billion in 1992 dollars. Asthma thus accounts for about 1% of all U.S. health care costs.

Bronchial Hyperreactivity

The other great changes in our conception of asthma derived from studies of airway responsiveness. It has been known for more than 50 years that asthmatics respond to inhalation of very low doses of histamine or methacholine aerosol with intense bronchoconstriction (*12*). This is a persistent abnormality, easily demonstrable even in asthmatics who are free of symptoms and who have normal pulmonary function at the time of testing. The demonstration of the ubiquity of bronchial hyperresponsiveness among asthmatics (*13*) caused the concept of asthma as an episodic disease to give way to the concept of asthma as a chronic abnormality in airway responsiveness that causes intermittent symptoms.

Bronchial hyperresponsiveness has come to be regarded as fundamental to asthma's pathogenesis, not only because it is ubiquitous in patients with the disease but also because its degree correlates with asthma's clinical severity. Bronchial responsiveness has been found to increase before the disease appears and to regress only after the disease has entered clinical remission.

Bronchial Hyperresponsiveness and Airway Mucosal Inflammation

Bronchial responsiveness was thought to be a fixed, possibly genetically determined function until it was found that increases in responsiveness could be induced in healthy people by viral respiratory infections or by brief exposure to ozone (*14,15*). Because both are inflammatory stimuli, these findings prompted speculation that bronchial responsiveness was somehow linked to airway mucosal inflammation (*16*). This idea has spawned many studies, but the study that had perhaps the greatest ultimate impact on theories of asthma's pathogenesis was the report that bronchial responsiveness increases for as long as 7 to 12 weeks following a single challenge with allergen in asthmatic subjects, particularly in those who develop a late asthmatic response 4 to 8 hr after the antigen challenge (*17*).

To determine whether the increase in responsiveness caused by antigen challenge is associated with airway mucosal inflammation, investigators have examined bronchial lavage obtained at the time of the late response (*18,19*). The fluid recovered contains increased numbers of eosinophils and polymorphonuclear leukocytes (PMN), just as are found in biopsies of skin taken at the time of the late cutaneous response to antigen injection (*20*).

The next step was logical. If the acute increase in responsiveness caused by antigen challenge is associated with an influx of inflammatory cells into the airways, is the hyperresponsiveness of ordinary asthma in the baseline state also associated with the presence of inflammatory cells in the mucosa?

The best answer to this question has been provided by studies of airway mucosal biopsies obtained by bronchoscopy of healthy and asthmatic subjects (*21,22*). Theses have been quite consistent in showing disruption or desquammation of the epithelium, subepithelial deposition of collagen (causing the spurious appearance of thickening of the basement membrane), edema and eosinophilic and lymphocytic infiltration of the lamina propria, and hypertrophy or hyperplasia of submucosal glands and airway smooth muscle. The findings in biopsies of people with mild asthma differ in degree from those found in patients dying of status asthmaticus, and the demonstration that eosinophilic inflammation is found even in mild asthma has focused attention on the eosinophil as playing a pivotal role in the pathogenesis of asthma (*23*).

Role of the Eosinophil

A detailed morphologic study of biopsies from 8 healthy and 34 asthmatic subjects provided corroborating evidence of the eosinophil's importance (*23*). This study found a significant but unimpressive difference in the number of intra- and subepithelial eosinophils in the mucosal biopsies from the asthmatic subjects. The difference became more impressive when special stains were used for two of the granule proteins released by eosinophils that have been activated eosinophil cationic protein and eosinophil-derived neurotoxin. These proteins were found outside the eosinophils in the biopsies from the asthmatic subjects, suggesting cell activation and mediator release.

Further supporting the case for the importance of the eosinophil are the results of *in vitro* and animal studies that have shown that eosinophil cationic protein could cause all of the changes responsible for airflow obstruction: increased contractile responsiveness of airway smooth muscle, increased vascular permeability, and mucus secretion (*24*).

Role of the Lymphocyte

The other cell that attracted attention is the lymphocyte, especially because the irregular, ovoid shape of lymphocytes in the biopsies from asthmatics suggested that the cells might be activated.

Again, the most enlightening findings came from studies of airway mucosal biopsies. One such study used immunohistochemical methods to compare the number of cells expressing interleukin-2 (IL-2) receptors, (taken to represent activated lymphocytes) in biopsies of proximal airways from asthmatic subjects, from nonasthmatic atopic subjects, and from healthy subjects (*25*). At both levels of the airways, the number of IL-2-positive cells was significantly greater in the asthmatic subjects than in the other groups of subjects. The number of IL-2-positive cells correlated with the number of eosinophils found in the same tissue.

The mechanism underlying this correlation was suggested by another airway biopsy study that examined tissues by *in situ* hybridization for expression of mRNA for IL-5, a cytokine whose actions include stimulation of the growth and activation of eosinophils (*26*). Positive cells were found

only in the biopsies from the asthmatic subjects. These findings suggest that lymphocytes in asthmatic airways may somehow be activated to produce cytokines responsible for eosinophil growth, attraction, and activation.

More recent studies have suggested predominance of a particular subtype of lymphocyte in asthmatic airways. These studies have examined lymphocytes recovered from bronchial lavage fluid obtained from asthmatic and healthy subjects, analyzing the pattern of cytokine production by *in situ* hybridization. The findings demonstrate predominance of the T_{H2} subset in asthmatic subjects (*27*). This subset of T-helper lymphocytes is characterized by its production of a cluster of interleukins: granulocyte macrophage colony stimulating factor (GM-CSF) and interleukins 3, 4, 5, and 10. Interleukin-4 is important in directing plasma cells to make IgE and, with IL-10, stimulates mast cell growth. Interleukin-5 promotes *in vitro* survival and activation of eosinophils, enhances eosinophil adherence to endothelium, and produces eosinophilia *in vivo*. GM-CSF and IL-3 also contribute to eosinophil maturation and differentiation.

Recently, Kay's group (*28*) has provided further evidence of the importance of this family of lymphocytes. Using the same methods, they showed an increase in the number of cells expressing message for the T_{H2} pattern of interleukins in bronchoalveolar lavage fluid obtained 24 hr after antigen challenge. The number of CD4/CD25+ cells in the fluid obtained correlates both with the number of cells expressing message for the T_{H2} interleukins and with the number of eosinophils found.

Synthesis into Conceptual Model of Asthma

These findings have led to the formulation of a conceptual model for the pathogenesis of allergic asthma (*29*). The model proposes that in a sensitized subject, the sequence starts with exposure to an antigen. The antigen binds to IgE on mast cells, and perhaps on macrophages or other cells, provoking secretion of the mediators of immediate hypersensitivity like histamine, prostaglandin D2, leukotrienes, and tryptase. In animal models, these agents cause many of the changes associated with acute asthma: marked, reversible narrowing of the airways from contraction of airway smooth muscle, engorgement of the mucosal vasculature, and mucosal edema.

To account for the late response, this model proposes that the antigen is presented to a T-helper lymphocyte, provoking it to produce interleukins that attract eosinophils and polymorphonuclear leukocytes and activate macrophages; these cells in turn release products that stimulate smooth-muscle contraction, edema, and mucus hypersecretion. The model also proposes that repeated activation of lymphocytes by repeated exposure to antigen accounts for chronic stimulation of the migration and activation of eosinophils in the airway mucosa, increasing airway responsiveness and causing the repeated bronchoconstriction typical of asthma.

A point where this model may already be out of date is in failing to recognize that the mast cell is itself a potential source of interleukins. Mast cells can synthesize interleukins 3, 4, 5, 6, 8, tumor necrosis factor, GM-CSF, and interferon-gamma (*30*), and are potentially responsible for all of the activities attributed to lymphocytes in this model.

This model offers many potential sites for therapeutic intervention ranging from antigen avoidance to stabilizing the mast cell, inhibiting the smooth muscle contraction, edema, and mucus secretion provoked by mast cell derived mediators, destroying lymphocytes or inhibiting their production of interleukins, antagonizing the action of the interleukins produced, preventing the attachment or migration of inflammatory cells, and, finally, to blocking the production or action of the mediators released from inflammatory cells. Of these possibilities, the most elegant is to prevent the entire cascade of events at the earliest possible point at the lymphocyte itself.

One of the agents most widely used for treating asthma may in fact act through inhibition of lymphocytes. Corticosteroids have long been known to reduce lymphocyte numbers in the circulation and have lately been shown to inhibit lymphocyte production of interleukins (*31*); studies of the effectiveness of corticosteroid therapy in asthma might be interpreted as evidence of the value of lymphocyte-directed therapy. Many clinical trials have indeed confirmed the efficacy of inhaled steroids in reducing bronchial reactivity and in improving symptoms of asthma (*32–34*). The National Institute of Health's National Asthma Education Program recommends the early use of inhaled corticosteroid for asthmatic patients with symptoms not controlled by occasional use inhaled beta agonists (*35*).

Limitations of the Conceptual Model

Most of the studies that have examined the validity of the conceptual model just described have relied on the studies of clinical, physiologic, or morphologic changes provoked by antigen challenge. Most attacks of asthma presenting to hospital emergency departments however cannot be traced to exposure to allergens, but instead appear to follow viral respiratory infections (*36*). That this distinction may be important was suggested by our own unexpected findings in a simple descriptive study of the constituents of the sputum spontaneously produced by asthmatic patients presenting for treatment to one of two large urban hospital emergency departments (*37*).

Our analysis focused on the cellular and chemical composition of the sputum. Whereas the current model of asthma's pathogenesis predicts that the predominant cell would be the eosinophil, we found that in the sputum produced by the 18 subjects we studied, the predominant cell is the PMN, not the eosinophil. In five of the sputum samples, more than 90% of the cells were PMNs, and in the samples from all but three subjects, more than 50% of the cells were PMNs. In only three subjects did the sputum match the pattern predicted from the conceptual model. In these samples, more than 75% of the cells were eosinophils and fewer than 10% were PMNs. None of these three patients recalled a viral respiratory infection as preceding their attack; of the five with more than 90% PMNs, four recalled such a preceding illness.

The chemical profile was also different. We found marked elevations of two interleukins produced by airway epithelial cells: interleukin-8, a potent chemoattractant for PMNs, and in IL-6, an interleukin whose actions are not well understood but that include inhibition of endotoxin-induced inflammation in rats. We could not detect interleukin-4 or GM-CSF in these samples.

Also elevated markedly was the concentration of neutrophil elastase. This serine protease is released from PMNs and potently stimulates submucosal gland secretion (*38*). The high level of elastase activity may thus account for the high concentrations of mucin, a product of goblet and mucus cell secretion that we also found in the samples we studied. Finally, albumin, a marker of vascular extravasation, was also increased in the samples.

These are just descriptive findings, but they fit well with the mechanism proposed for the pathogenesis of mucus hypersecretion in cystic fibrosis and bronchiectasis—that mucus hypersecretion is the end result of an inflammatory cascade that begins with airway epithelial injury. This injury stimulates IL-8 secretion and thus the chemoattraction. Somehow, neutrophils are activated to secrete elastase that in turn stimulates mucin secretion from airway goblet cells and submucosal glands (*39*).

At present, this line of reasoning is speculative, but the point is not so much whether it is correct but that it is only possible because of findings made in asthma as it occurs in the real world, where attacks appear to be triggered most often by viral infection. This is not to say that the model derived from studies of the response to antigen is irrelevant, it is rather to say that it is incomplete. Somehow, the mechanisms of response to antigen inhalation that are in place in the asthmatic airway interact with the mechanisms of response to viral infection or other airway insult to provoke the events of acute severe attacks. How these systems of response interact may be the next major research issue for those interested in asthma.

Results

Possible Effects of Air Toxics in Asthmatic Patients

Other papers presented at this symposium directly consider the evidence that air toxics have important adverse effects in asthmatic patients. The conceptual model of asthma predicts several possible mechanisms of interaction. One such mechanism has to do with allergic sensitization of the airways themselves. A genetically determined disposition is clearly important, and the concentration of certain allergens in indoor air appears to be an important co-factor (*10*). The observation of a strong influence of maternal smoking as an additional factor suggests that inhaled air pollutants and air toxics may alter the integrity of the airway epithelial barrier, increasing the ease of sensitization to inhaled allergen (*40*). This possibility is supported by studies of murine and canine models in which concurrent ozone exposure has been shown to facilitate sensitization to an inhaled allergen (*41,42*). Greater penetration of allergen may account for the ozone's enhancement of the response of allergic subjects to intranasal instillation of allergen (*43*).

It should have been no surprise that pollutants that cause bronchoconstriction when inhaled in high concentration by healthy subjects should provoke intense bronchoconstriction even when inhaled in low concentration by asthmatic subjects; bronchial hyperresponsiveness to nonspecific inhaled stimuli has long been known to be associated with asthma. This has been confirmed repeatedly to be the case for sulfur dioxide; asthmatics respond to an order of magnitude lower concentrations of SO_2 than do nonasthmatics (*44,45*). This may account for the association of asthmatic symptoms and atmospheric concentrations of sulfates in epidemiologic studies (*46*). It would be easier to make predictions about which other inhaled materials should cause bronchoconstriction if we had a better idea of the mechanisms by which SO_2 produces this effect.

The study of whether and how inhaled materials provoke cytokine release in the airways, as ozone has been shown to do from airway epithelial cells (*47*), has just begun. That these important mediators of cell to cell communication may have important, even profound effects on airway function seems certain. As their cellular sources and effects are better delineated, it will become easier to design *in vitro* and animal studies of the effects of air toxics that predict effects in subjects in controlled exposure experiments and perhaps even in population studies.

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